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General Introduction
Chronic Obstructive Pulmonary Disease (COPD) is currently the third leading cause of death worldwide and the prevalence is expected to rise even further. COPD patients often suffer from comorbidities including lung cancer, osteoporosis, skeletal muscle dysfunction, depression, gastrointestinal diseases, metabolic syndrome and cardiovascular diseases (CVD) in addition to the pulmonary features. Especially CVD, mainly caused by atherosclerosis, is an important cause of morbidity and mortality in COPD patients. Atherosclerosis is a disease of the large arteries, and dyslipidemia and systemic inflammation are important contributors to atherogenesis. COPD patients have an increased risk to develop atherosclerosis compared to matched controls, even after correction of common risk factors such as smoking. Smoking cessation is important to limit the progression of the disease, however, even after smoking cessation there is a considerable higher risk to develop COPD and atherosclerosis. There are several mechanisms which may explain the link between COPD and atherosclerosis, including dietary habits and dyslipidemia, infection with microorganisms, systemic inflammation, oxidative stress and endothelial dysfunction. Recent data indicate that brown adipose tissue (BAT) contributes significantly not only to lipoprotein metabolism, but activation of BAT also may inhibit atherosclerosis development. Mouse models provide good platforms to study either COPD or atherosclerosis, but also atherosclerosis and COPD combined. Therefore, in this thesis we focussed on mouse models for COPD and CVD, and their use in development of new treatments.

1.1 Chronic obstructive pulmonary disease (COPD)

1.1.1 Pulmonary structure and function
The respiratory tract consists of the upper and lower respiratory tract. The upper tract starts at the nose, and includes the nasal passages, paranasal sinuses, pharynx and part of the larynx. The lower airways consist of the trachea, bronchi and bronchioles, and the respiratory zone including the respiratory bronchioles, alveolar ducts, alveolar sacs and alveoli. The conducting zone consists of the upper tract and part of the lower respiratory system and mainly functions in the filtration, warming and moistening of inhaled air. The respiratory zone, consisting of the respiratory bronchioles and alveoli, functions in exchange of inhaled oxygen and collected carbon dioxide (CO$_2$) from the blood (1).

The whole respiratory tract is covered with airway epithelial cells including ciliated cells, secretory club cells, neuroendocrine brush cells, mucus-producing goblet cells and basal cells (Figure 1). The airway epithelial lining is in direct contact with inhaled air at the luminal side and serves as a physical primary barrier against inhaled pathogens and particles. Goblet cells produce mucus in order to trap inhaled dust, particles and microorganism. Cilia on the apical side of ciliated cells sweep foreign material that is entrapped in the mucus layer.
from the lungs in a concerted beating manner. This process of mucociliary clearance is an important defence mechanism of the airway epithelium. Basal cells reside at the basal side of the epithelium and act as precursor cells for other cell types and can repopulate the epithelial layer after injury and differentiate towards the different epithelial cell types. Secretory club cells secrete surfactant-related proteins, which play a role in host defence and are necessary during respiration and are present in higher numbers towards the alveoli. Epithelial cells also line the alveoli, and are referred to as alveolar type I and II cells. Alveolar type II cells produce surfactant and act as repopulating cells for the type I cells, which are involved in gas exchange. Other cell types are airway smooth muscle cells located circumferentially around the airways and fibroblasts providing mechanical, structural and biochemical support enabling bronchodilation and constriction (1).

In addition to acting as a primary barrier and the mucociliary clearance apparatus, epithelial cells provide several other defence mechanisms (2). Intruded microorganisms can be recognized by epithelial cells and other immune cells through pattern-recognition receptors, including several Toll-like receptors (TLRs), activation of which causes activation of intracellular signaling pathways, leading to production and secretion of (pro-) inflammatory mediators. TLR4, for example, recognizes lipopolysaccharide (LPS), present on the surface of Gram-negative bacteria. Epithelial cells also respond to microbial triggering by production of antimicrobial peptides and proteins including defensins, which are important in microbial elimination. In addition to the defensive response of epithelial cells, there are more specialized immune cells present in the lungs including alveolar macrophages, dendritic cells and natural killer (NK) cells.

Pathogens can be phagocytised by alveolar macrophages. Furthermore, macrophages and epithelial cells produce pro-inflammatory cytokines and chemokines such as Interleukin-6 (IL-6), IL-8, IL-12, Tumor Necrosis Factor-α (TNF-α) and Monocyte Chemoattractant Protein-1 (MCP-1) to recruit other inflammatory cells. Recruited cells comprise neutrophils, monocytes, eosinophils and lymphocytes. The inflammatory cells produce cytokines and proteolytic factors aimed to eliminate the intruded pathogen, including (neutrophil) elastase, matrix metalloproteases (MMPs), cytokines and chemokines. This innate immune response serves as a direct response to pathogen intrusion. If this response is insufficient to eliminate the pathogen and/or if inflammation persists, the adaptive immune system is activated. Antigen presenting cells, such as dendritic cells, can process pathogen molecules, migrate to draining lymph nodes and present the pathogenic antigens to B and T lymphocytes (3, 4). These cells display a more specialized and specific immune response towards the pathogen and can provide a memory response (5), which will not be discussed here.
Figure 1: Simplified overview of the airway epithelium in health and in COPD

A) Normally, the airways are lined with epithelial cells, consisting of various cell types, including ciliated cells, club cells, mucus-producing goblet cells and basal cells. Cilia on ciliated cells within the periciliary layer beat in a concerted manner to remove mucus and entrapped particles/microorganisms from the lungs (mucociliary clearance apparatus). Smooth muscle cells provide support and enable contraction of the airways. Extracellular matrix components are produced by fibroblasts, smooth muscle cells and epithelial cells. Epithelial cells and macrophages are important for sensing of pathogens, which can then be cleared by macrophages. B) In lungs of COPD patients, there is mucus hypersecretion by goblet cells. Furthermore, cilia length is shortened, which impairs ciliary functioning. This results in an impaired mucociliary clearance of inhaled pathogens and particles, increasing the risk for infection. In response to cytokines and growth factors, smooth muscle cells and fibroblasts proliferate and contribute to excessive extracellular matrix deposition. On top of that, circulating leukocytes are recruited, which further increases inflammation.
1.1.2 COPD pathophysiology

The lungs are important for respiration, to provide our body with oxygen, and contain specialized cells to minimize infiltration of inhaled particles and micro-organisms as described in section 1.1.1. However, COPD is a condition in which both the lung function and the lung defense mechanism are impaired. COPD is the third leading cause of death worldwide (Figure 2) and the prevalence has not decreased over the past decades and is even expected to rise in the coming years (6). COPD is defined as a common preventable disease characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. The most important risk factor for COPD is cigarette smoking, although air pollution, in-house cooking, and genetic predisposition may enhance the risk of COPD development (7). Approximately 10-20% of smokers develop COPD, which may partly be explained by genetic predisposition. A well-known genetic predisposition is hereditary α-1-antitrypsin deficiency, where patients develop COPD at a relatively young age compared to COPD patients without this deficiency (8). The protein α-1-antitrypsin is produced in the liver and is an inhibitor of serine proteases, including neutrophil elastase (NE). NE degrades alveolar elastin and induces mucus hypersecretion in the lung, and is increased in the lungs of COPD patients.

![Figure 2: COPD prevalence worldwide over the last few years](http://www.who.int/mediacentre/factsheets/fs310/en/)

A) The top 4 leading causes of death worldwide during the past decade are ischaemic heart disease, stroke, lower respiratory infections and COPD. B) Whereas the rate of COPD-related deaths remained similar in the period 2000-2012, many COPD patients die from comorbidities such as cardiovascular disease and lung cancer that have increased in the same period. Tobacco smoking is a major cause of many of diseases with high mortality, including CVD, COPD and lung cancer. In total, tobacco use is responsible for the death of about 1 in 10 adults worldwide (6). Reprinted with permission from: The top 10 causes of death, WHO Fact sheet Nº310, (2015) http://www.who.int/mediacentre/factsheets/fs310/en/
One of the most important causes of COPD is cigarette smoking. Cigarette smoke (CS) contains over 5,000 components, of which many are also present in air pollutants. These components have direct and indirect effects on various organs. The addicting effect of cigarettes is mediated by nicotine, which acts on nicotinic receptors in the brain, however is also expressed on various other organs and cell types, including endothelial cells lining the arteries. Acrolein and acetaldehyde in CS cause pulmonary effects, whereas cyanide and arsenic increase the risk for COPD development. Also metals, polycyclic hydrocarbons, other chemicals and LPS in CS contribute to the pathogenesis of lung-related diseases and COPD comorbidities (9). In lungs of COPD patients, repeated exposure to noxious particles and gases, for example from CS, directly impair the barrier function of the airway epithelial cells and mucociliary clearance of foreign substances (Figure 1), increasing the risk for infiltration and infection. Upon exposure to CS components, epithelial cells and macrophages are activated and produce transforming growth factor-β (TGF-β). In COPD, a continuous production of TGF-β induces fibroblast proliferation and fibrosis of the small airways. Macrophages produce reactive oxygen species (ROS), cytokines, chemotactic factors and proteolytic enzymes. Chemotactic factors induce recruitment of circulating leukocytes, inducing an ongoing inflammatory process. The recruited and resident immune cells release proteolytic enzymes such as MMPs, causing degradation of alveolar elastin, leading to development of emphysema. Neutrophils produce elastase, which induces excessive mucus production by goblet cells, leading to mucus hypersecretion, and also degrades elastin. These three hallmarks of COPD, fibrosis, emphysema and mucus hypersecretion, due to an exaggerated inflammatory response, lead to obstructive airway disease.

1.1.3 COPD severity and treatment
The level of airway obstruction and severity of COPD are based on the lung function parameter forced expiratory volume in 1 second (FEV$_1$), the amount of air which is maximally exhaled in 1 second. Due to differences in lung volume, this parameter is corrected for the forced vital capacity (FVC), the maximal volume of air which can be exhaled. COPD severity ranges from stage 1 (mild disease) till stage 4, the most severe stage (7). In addition to an exaggerated response towards noxious particles and gases, COPD patients also have an increased risk for respiratory infections and an altered airway microbiome (10), with increased levels of pathogenic micro-organisms, such as *Haemophilus influenzae* and *Pseudomonas aeruginosa* (11). Although COPD classification is based on the level of airflow obstruction primarily, exacerbations and comorbidities contribute to the overall disease severity in individual patients. Exacerbations, a sudden worsening of the symptoms often triggered by infections, for example, are associated with increased circulating levels of C-reactive protein (CRP) and Serum Amyloid A (SAA) (12, 13), which contribute to systemic inflammation in COPD patients, leading to increased morbidity and mortality. Most COPD patients are currently treated with
long-acting bronchodilators such as long-acting β2-adrenergic receptor agonists (LABAs) and long-acting muscarinic receptor antagonists (LAMAs) to facilitate breathing, together with inhaled steroids to limit the extent of inflammation. However, a large group of COPD patients do not respond to inhaled steroids well. Furthermore, upon exacerbations, COPD patients often require systemic corticosteroids and additional antibiotics.

As described in the beginning of this chapter, comorbidities of COPD include lung cancer, osteoporosis, skeletal muscle dysfunction, depression, gastrointestinal diseases, metabolic syndrome and cardiovascular diseases (CVD). Especially CVD, with atherosclerosis as the main underlying cause, in COPD patients is an important cause of morbidity and mortality (14). Although smoking is an important contributor to both COPD and CVD, it does not explain the increased risk for CVD in COPD patients. Furthermore, even after smoking cessation, there is still an increased risk for both COPD and CVD development compared to never-smokers (15). One of the main mechanisms that is important in the link between COPD and associated comorbidities is systemic inflammation (16, 17). This may arise from leakage of pro-inflammatory mediators from the lung to the circulation or from inhaled nanoparticles present in CS that reach the circulation. However, other mechanisms that link COPD and CVD include hypoxia, dyslipidemia and CS-generated ROS, which may lead to endothelial injury. Treatment of the comorbidities of COPD patients depends on the stage, severity of COPD and is patient specific. Although in general, the comorbidities of COPD are treated as a separate modality, there may be some interplay between different drugs, which has not yet been studied in detail. Some of the mechanisms that link COPD and CVD and an overview of combined murine models of COPD and CVD are described in Chapter 2.

1.1.4 Mesenchymal stromal cells as potential treatment for COPD and CVD

Mesenchymal stromal cells (MSC), also known as mesenchymal stem cells, have emerged as potential anti-inflammatory and regenerative cell therapeutic tool over the last decade (18). MSC are defined as spindle-shaped, plastic adherent cells, with multipotent differentiation capacity *in vitro* into adipocytes, chondrocytes and osteoblasts in presence of the appropriate (growth) factors. Although there is much debate on the identification and culturing of these cells, there is a consensus on their identification based on the absence of the hematopoietic markers CD34, CD45, CD14/Cd11b, CD79α and the major histocompatibility complex II (MHCII) class receptor HLA-DR, whereas the surface markers CD105 (endoglin), CD73 (ecto 5’nucleotidase) and CD90 (Thy-1) are present on MSC (19). MSC can be isolated from different sources that harbour high concentrations of progenitor cells, such as bone marrow, blood, adipose tissue and placenta. There is also increasing evidence that MSC also reside in organs such as lung, heart, kidney and liver. These organ-derived MSC may be more potent in organ-specific actions and regeneration, however, the precise role of these cells in endogenous repopulation and regeneration upon injury and damage is not known yet.
Several research groups demonstrated various functions of MSC in vitro, although functional effects and level of maintenance after in vivo application are not very well known and should be studied in more detail. Although MSC have the capacity to differentiate into different cell types, it is commonly accepted nowadays that they mostly act via paracrine mechanisms (20). MSC produce growth factors, cytokines and chemokines including IL-6, granulocyte colony stimulating factor (GSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF). MSC can execute their immunomodulatory and regenerative role at different levels. By secretion of trophic or paracrine factors, MSC can induce angiogenesis, protect from apoptosis, induce proliferation and induce recruitment of endogenous stem- or progenitor cells. MSC may transfer vesicles containing mRNA, proteins, microRNA or mitochondria in order to influence the host cells. Furthermore, they can inhibit pro-inflammatory responses by inhibition of maturation of antigen presenting cells (dendritic cells and macrophages), suppression of antibody production, inhibition of proliferation and activity of inflammatory cells such as neutrophils, NK cells, CD4+, CD8+, and B lymphocytes, and induce regulatory cells such as regulatory T cells (21).

Although the precise mechanism of action of MSC is not known, the effect of MSC treatment has been examined in phase I clinical trials of (chronic) inflammatory diseases such as Graft-versus-host-disease, inflammatory bowel disease, but also COPD and CVD. MSC therefore are promising as cell therapy in (chronic) inflammatory diseases and may therefore be used as therapy for COPD and CVD, which we studied in Chapter 4.

1.2 Atherosclerosis

1.2.1 Lipoprotein metabolism

One of the main energy sources in our body and organs are fatty acids (FA). Our body enables continuous storage and utilization of FA through lipoprotein metabolism (Figure 3). Within the circulation, lipids are transported in lipoprotein particles, with different ranges in density and size, and are known as chylomicrons, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (Table 1). Lipoproteins have a lipid-rich core containing triglycerides (TG) and cholesteryl esters surrounded by an amphiphilic outer surface containing phospholipids, unesterified cholesterol and apolipoproteins. TG in TG-rich lipoproteins (chylomicrons, VLDL and LDL) are hydrolysed by lipoprotein lipase (LPL) on endothelial cells of metabolically active organs. The resulting FA can be taken up by skeletal muscle or the heart, to be used as energy source, stored as TG in white adipose tissue (WAT), or oxidized in brown adipose tissue (BAT) to generate heat (Figure 3). Chylomicron remnants are taken up by binding of apolipoprotein E (ApoE) to the LDL-receptor (LDLr), the LDLr-related protein (LRP), or proteoglycans on hepatocytes. The liver can endogenously pack TG and cholesterol into VLDL. Similar to chylomicrons, VLDL release FA upon LPL-mediated
hydrolysis, resulting in the formation of VLDL remnants that can also be taken up by the liver via ApoE. If hepatic uptake does not occur, these VLDL remnants can be completely lipolysed resulting in the formation of LDL, which is able to transport cholesterol from to peripheral organs. VLDL remnants and LDL contain relatively high levels of cholesterol and are therefore pro-atherogenic lipoproteins. Cholesterol can be transported back to the liver for excretion in bile, through a process known as reverse cholesterol transport (RCT), where cholesterol from peripheral organs are transferred via HDL and taken up by the liver via scavenger receptor BI (SR-BI) (22). Furthermore, cholesterol can also be exchanged from VLDL/LDL to HDL through the enzyme cholesteryl ester transfer protein (CETP), which will not be further discussed here.

### Table 1. Content and size of circulating lipoprotein particles

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Protein</th>
<th>Triglycerides</th>
<th>Phospholipids</th>
<th>Cholesteryl esters</th>
<th>Cholesterol</th>
<th>Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons</td>
<td>1-2%</td>
<td>85-88%</td>
<td>~8%</td>
<td>~3%</td>
<td>~1%</td>
<td>100-500</td>
</tr>
<tr>
<td>VLDL</td>
<td>5-12%</td>
<td>50-55%</td>
<td>18-20%</td>
<td>12-15%</td>
<td>8-10%</td>
<td>30-80</td>
</tr>
<tr>
<td>LDL</td>
<td>20-22%</td>
<td>10-15%</td>
<td>20-28%</td>
<td>37-48%</td>
<td>8-10%</td>
<td>18-28</td>
</tr>
<tr>
<td>HDL</td>
<td>55%</td>
<td>3-15%</td>
<td>26-46%</td>
<td>15-30%</td>
<td>2-10%</td>
<td>5-12</td>
</tr>
</tbody>
</table>

#### 1.2.2 Atherosclerosis

Ischaemic heart diseases are the leading cause of death worldwide as shown in Figure 2, and their prevalence has increased rapidly over the last years. The most important cause is a sedentary lifestyle and increased dietary lipid intake, also known as the Western lifestyle. Normally, intake of lipids is necessary to supply our organs with energy and there is a clear balance between energy intake and energy utilization. In modern lifestyle, however, many people suffer from an unbalanced energy state due to increased caloric uptake and lower energy expenditure. This unbalance expresses itself in increased circulating levels of the pro-atherogenic lipoproteins VLDL and LDL.

Atherosclerosis occurs mainly in the large arteries. The luminal side of arteries is aligned by the intima, comprised of mainly endothelial cells, which is surrounded by the tunica media containing smooth muscle cells, enabling vasoconstriction and dilation. The media is in its turn surrounded by the adventitia which contains fibroblasts, mast cells and microvessels, supplying blood to the large arteries (Chapter 2, Figure 2). Upon injury to the endothelium, for example by oxidative stress, the endothelium becomes more adhesive for leukocytes, which can protrude into the vascular wall, forming the neointima. Oxidative stress also modifies LDL into oxidized LDL (oxLDL) which can be recognized by monocytes (17). High levels of LDL and VLDL increase the risk for endothelial injury, which enables the uptake of atherogenic lipoproteins by macrophages. Upon protrusion into the vessel wall, monocytes differentiate into macrophages, and take up modified lipoproteins that induces formation of foam cells. Activated macrophages and foam cells produce pro-inflammatory
mediators, including monocyte chemoattractant protein (MCP)-1, which recruits other leukocytes towards the developing plaque. Furthermore, smooth muscle cells from the media migrate towards the intima, where they proliferate and produce collagen and elastin. Due to growing of the plaque, the inner core becomes hypoxic, resulting in formation of a necrotic core containing necrotic and apoptotic cells, covered by a fibrous cap produced by smooth muscle cells. The necrotic core in the most severe form also contains cholesterol crystals. Upon stress, the fibrous cap can rupture, followed by spilling of pro-thrombotic mediators into the vessel lumen and thrombus formation. This process can occlude the lumen as occurs during myocardial infarction or stroke (23, 24).

Figure 3: Simplified overview of lipoprotein metabolism in the human body
Dietary lipids taken up in the intestine are mainly transported in triglyceride (TG)-rich chylomicrons. TG can be hydrolysed by lipoprotein lipase (LPL), leading to the release of fatty acids (FA). FA acts as fuel for organs such as the heart and skeletal muscle, can be stored in white adipose tissue (WAT) depots and ectopically in organs including the liver, or can be combusted by brown adipose tissue (BAT), thereby releasing heat. Chylomicron remnants are taken up by the liver by binding of apolipoprotein E (ApoE) to low-density lipoprotein receptors (LDLr), LRP and proteoglycans on hepatocytes. TG and cholesterol can endogenously be packed into very-low-density-lipoproteins (VLDL) in the liver. These particles release FA in the circulation upon encountering LPL, generating VLDL remnants and ultimately forming low-density-lipoprotein (LDL). LDL transports cholesterol to peripheral organs, but after oxidation (for example by oxidative stress from CS), can also be taken up by macrophages and cause atherosclerosis. Cholesterol can be taken up from peripheral organs by high-density-lipoprotein (HDL) and be transported back to the liver for secretion into bile.
1.2.3 Brown adipose tissue in lipoprotein metabolism and CVD

Brown adipose tissue (BAT) functions in non-shivering thermogenesis and was classically known to be present between the shoulders of mammalian newborns. However, it is now clear that human adults also have BAT depots around the aorta, supraclavicular and dispersed within white adipose tissue (WAT) depots (25). In contrast to white adipocytes, which contain a large lipid droplet and few mitochondria, brown adipocytes contain numerous small lipid droplets and a high number of mitochondria (Figure 4), being responsible for the brownish colour of the tissue. In contradiction to WAT, to which blood supply is relatively low, BAT contains many capillaries and is well supplied with blood.

![Figure 4. HE staining of white and brown adipose tissue](image)

A) White adipose tissue is characterized by large lipid droplets and low number of mitochondria, and is mainly responsible for fat storage, whereas B) brown adipose tissue is characterized by numerous small lipid droplets and many mitochondria, aimed at combustion of fat to generate heat.

Brown adipocytes are activated through the sympathetic nervous system (SNS), for example after cold sensation, which activates the ventromedial hypothalamic nucleus in the brain. Upon SNS activation, sympathetic neurons release catecholamines, including the neurotransmitter norepinephrine (NE), which binds adrenergic receptors on brown adipocytes (26). In mice this is mediated primarily by β3-adrenergic receptors, whereas in humans this may also be mediated through α-β-adrenergic receptors, which is still being investigated in more detail. Alternatively activated macrophages can also release NE locally and activate BAT (27). Activation of the β3-adrenergic receptors results in adenylyl cyclase-mediated increase in cyclic adenosine monophosphate (cAMP) and activation of protein kinase A (PKA). PKA phosphorylates downstream signaling molecules leading to activation of the transcription factor Atf2 which binds a DNA-binding complex with PPAR-γ, PPAR-α and retinoid X receptor. This downstream signalling cascade facilitates the transcription of the thermogenic target gene uncoupling protein 1 (UCP-1), which is the classical marker for brown adipocytes. Activation of PKA also results in intracellular lipolysis, releasing
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FA from intracellular lipid droplets to be combusted by mitochondria. The released FA can bind to UCP-1 which uncouples electron transport from ATP production, resulting in heat production (thermogenesis) (25). Although it was known that BAT takes up FA from circulating lipoproteins (28) to replenish intracellular FA stores, the precise mechanism of uptake is unknown. Therefore, we studied the mode of FA uptake from the circulation into BAT in more detail in Chapter 5. Several mouse studies showed that BAT activation is increased upon cold exposure in mice and alters levels of pro-atherogenic lipoproteins (28). However, to which extent BAT contributes to lipoprotein metabolism in humans is still being investigated. Finally, BAT activation may be targeted to lower pro-atherogenic lipid levels and therefore lower CVD. Therefore, in Chapter 6, we determined whether BAT activation can be targeted for the treatment of CVD.

1.5. Outline of thesis

COPD patients have a chronic inflammatory lung disease, which is often associated with an increased risk for atherosclerosis underlying CVD. Although systemic inflammation is suggested to link these two chronic diseases, the precise mechanism of the increased risk to develop atherosclerosis is not known yet. In chapter 2, we reviewed whether combined murine models of COPD and CVD represent human pathophysiology well. Furthermore, using these combined models, we present an overview of the mechanisms that may explain the link between COPD and CVD. In order to understand the link between COPD and CVD better, chapter 3 describes the effect of emphysema alone and emphysema with chronic LPS-induced pulmonary inflammation on atherosclerosis development in APOE*3-Leiden (E3L) mice. The E3L atherosclerosis model was also used to examine the effect of short- and long-term treatment with MSC on LPS-induced pulmonary inflammation, emphysema and atherosclerosis in chapter 4. Smoking is the most important risk factor for COPD, but also important in atherosclerosis development. Smoking is associated with lower body weight and increased energy expenditure, which may be caused by BAT activation. Although BAT research has gained interest only in the last few years, it is now clear that BAT activation prevents obesity, lowers lipid levels, and contributes to lipoprotein metabolism. Therefore, we examined the mode of FA uptake from the circulation in BAT in Chapter 5. Subsequently, we examined whether BAT activation, through β3-adrenergic receptor agonism, could lower pro-atherogenic lipid levels and atherosclerosis in various atherosclerosis models in Chapter 6. The most important findings of the studies described in this thesis, the clinical implications and future perspectives of our findings are discussed in Chapter 7.
Chapter 1

References

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